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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. ER 446086692 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Jonathan	ROTH	San Diego, CA
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Additional inventors are being named on the ____________ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHODS FOR AFFECTING BODY COMPOSITION

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☑ Specification *Number of Pages* 21

☑ Drawing(s) *Number of Sheets* 12

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number ____________

☐ Other (specify) ____________

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Respectfully submitted,

SIGNATURE [signature]

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Date March 4, 2004

REGISTRATION NO. 44,830
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030404 U.S. PTO 1607

FEE TRANSMITTAL for FY 2004

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☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 160.00

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1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
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1004	770	2004	385	Reissue filing fee	
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2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

			Extra Claims		Fee from below		Fee Paid
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Independent Claims		- 3** =		X		=	
Multiple Dependent						=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

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FEE CALCULATION (continued)

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1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) ____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY				(Complete *(if applicable)*)	
Name *(Print/Type)*	Mi Kim	Registration No. (Attorney/Agent)	44,830	Telephone	858 552-2200
Signature	[signature]			Date	March 4, 2004

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METHODS FOR AFFECTING BODY COMPOSITION

FIELD OF THE INVENTION

The present invention relates to the fields of medicine, health and nutrition.

BACKGROUND OF THE INVENTION

It is estimated that about 64% of Americans are overweight or obese (roughly about 97 million adults) and it is generally believed that it is only getting worse. Being obese or overweight substantially increases the risk of morbidity from hypertension; dyslipidemia; type 2 diabetes; coronary heart disease; stroke; gallbladder disease; osteoarthritis; sleep apnea and respiratory problems; and endometrial, breast, prostate, and colon cancers. Higher body weights are also associated with increases in all-cause mortality.

In humans, patients who are overweight or obese are considered those with a Body Mass Index (BMI) of equal or greater than 25. BMI is a common measure expressing the relationship (or ratio) of weight-to-height. It is a mathematical formula in which a person's body weight in kilograms is divided by the square of his or her height in meters (*i.e.*, $wt/(ht)^2$. Individuals with a BMI of 25 to 29.9 are considered overweight, while individuals with a BMI of 30 or more are considered obese.

According to the NIH Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults, all adults (aged 18 years or older) who have a BMI of 25 or more are considered at risk for premature death and disability as a consequence of overweight and obesity. These health risks increase even more as the severity of an individual's obesity increases.

The trend towards increased obesity can be seen from 1985 to 2002, where there has been a dramatic increase in obesity in the United States. In 1985 only a few states were participating in CDC's Behavioral Risk Factor Surveillance System (BRFSS) and providing obesity data. In 1991, four states were reporting obesity prevalence rates of

15–19 percent and no states reported rates at or above 20 percent. In 2002, 20 states have obesity prevalence rates of 15–19 percent; 29 states have rates of 20–24 percent; and one state reports a rate over 25 percent.

Because obesity has risen at an epidemic rate during the past 20 years, one of the national health objectives for the year 2010 is to reduce the prevalence of obesity among adults to less than 15%. However, research indicates that the situation is worsening rather than improving.

A recent study by researchers at RTI International and the CDC estimates that U.S. obesity-attributable medical expenditures reached $75 billion in 2003 and that taxpayers finance about half of these costs through Medicare and Medicaid. Total state-level expenditure estimates in 2003 dollars range from $87 million in Wyoming to $7.7 billion in California. Obesity-attributable Medicaid expenditure estimates range from $23 million in Wyoming to $3.5 billion in New York. Medicare expenditures range from $15 million in Wyoming to $1.7 billion in California.

Existing therapies for obesity include standard diets and exercise, very low calorie diets, behavioral therapy, pharmacotherapy involving appetite suppressants, thermogenic drugs, food absorption inhibitors, mechanical devices such as jaw wiring, waist cords and balloons, and surgery, such as gastric bypass. Jung and Chong, *Clinical Endocrinology*, 35:11-20 (1991); Bray, *Am. J. Clin. Nutr.*, 55:538S-544S (1992).

In general, while loss of fat is desired, loss of lean body mass, for example, proteins, is not. Lean body mass is highly active metabolically and physiologically and the size is genetically defined and maintained. Lean body mass contains all the body protein. There is no real protein store as every protein molecule has a role in maintaining homeostasis. It is believed that loss of body protein is deleterious to the health of an individual. The majority of the protein in the lean body mass is in the skeletal muscle mass. Lean body mass is 50-60% muscle mass by weight, the rest is bone and tendon. Protein makes up the critical cell structure in muscle, viscera, red cells and connective tissue. Enzymes, which direct metabolism, and antibodies, which maintain immune

function, are also proteins. Thus, it is desirable to prevent loss of lean body mass while reducing body fat.

Caloric restriction, regardless of its form, can cause catabolism of body protein and produce negative nitrogen balance. Protein-supplemented diets, therefore, have gained popularity as a means of lessening nitrogen loss during caloric restriction. Protein-sparing modified fasting has been reported to be effective in weight reduction in adolescents. Lee et al. *Clin. Pediatr.,* 31:234-236 (April 1992). However, these diets may produce only modest nitrogen sparing. A need exists for effective ways of promoting fat loss yet preserving lean body mass.

What are described herein are novel methods for modifying body composition.

SUMMARY OF THE INVENTION

In one general aspect, methods of the invention include modifying body composition, for example, reducing body fat, but not lean body mass. Methods for treating obesity using amylin and amylin agonists have been described in U.S. Patent Application No. 09/445,517, filed June 5, 1998, and U.S. Patent Application No. 08/870,762, filed June 6, 1997, the entire contents of which have been incorporated herein. However, it has surprisingly been discovered that amylin and amylin agonists may have a metabolic effect and may also be used to affect body composition, leading to the desirable loss of body fat, yet preserving lean body mass. Moreover, amylin did not induce tolerance/resistance in a subject when administered by osmotic pump, unlike sibutramine.

In certain embodiments, methods of the invention include reducing body fat or preventing body fat gain. Other embodiments include controlling body weight and/or sculpting a body's appearance. The subjects to whom these methods may be of interest are those individuals who are overweight or obese. However, subjects with lean body composition, such as body builders and other athletes, may benefit from the invention as well. It may be desirable for them to reduce or maintain their body weight, *e.g.,* to stay in

a certain weight class range, yet preserve or increase their lean body mass for greater strength, stamina, and/or a more muscular appearance.

In certain embodiments of the invention, administration of amylin or amylin agonist is done peripherally and not centrally, *i.e,* not through the central nervous system.

It is further contemplated that methods of the invention can be used in combination with other forms of nutritional regimens and weight loss programs, such as those already described above, for example, those that include life-style changes that include monitoring food intake (quantity and quality) and exercising, as well as including other diet drugs and surgery.

In yet another general aspect, methods of the invention can include the use of amylin and amylin agonists to reduce the fat content in animals for consumption. In other words, methods of the invention can include producing a leaner meat source.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims. All references cited herein are incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B depict the effects of chronic administration of amylin or sibutramine on food consumption and body weight, respectively, in DIO rats.

Figures 2A and 2B depict the body composition of DIO rats chronically administered with amylin or sibutramine, respectively.

Figures 3A and 3B depict the leptin, insulin and triglycerides levels of DIO rats chronically administered amylin or sibutramine.

Figures 4A and 4B show the effects on food consumption and body weight, respectively, of three differing doses of amylin in DIO rats.

Figure 5 depicts the effect of amylin on food intake in lean rats.

Figures 6A and 6B depict the effect of amylin on weight in lean rats.

Figure 7 depicts the effect of amylin on food intake in DIO Levin rats.

Figures 8A and 8B depict the effect of amylin on weight in DIO Levin rats.

Figures 9A-9F depict the triglyceride, cholesterol, glucose, insulin, leptin, and liver triglyceride levels in lean rats chronically administered amylin.

Figures 10A-10E depict the triglyceride, glucose, cholesterol, insulin, and leptin levels in DIO Levin rats chronically administered amylin.

Figures 11A-11D depict the tissue biochemistry of DIO Levin rats chronically administered amylin.

Figures 12A-12D depict the weight of selected fat pad as a percent of total body weight in DIO Levin rats chronically administered amylin.

DESCRIPTION OF THE INVENTION

It has now been discovered that amylin and amylin agonist, which include amylin agonist analogs and derivatives, may have metabolic effects on the body and may be used to preferentially reduce body fat and spare lean body mass.

The present invention is directed to controlling body weight by reducing body weight, maintaining body weight, or reducing body weight gain, while selectively reducing body fat or preventing body fat gain and maintaining or increasing lean body mass. In certain situations, however, it may be desirable to increase body weight, for example, through selective nutrient intake (*e.g.*, increasing the caloric or fat content), while reducing or maintaining percent body fat, *e.g.,* body building.

The methods of the invention contemplate the use of an effective amount of amylin or amylin agonist in the body to affect the desired results as described in the claimed methods.

The administered amylin or amylin agonist may be in the form of a peptide, a prodrug, or as pharmaceutical salts thereof. The term "prodrug" refers to a compound that is a drug precursor that, following administration, releases the drug *in vivo* via some chemical or physiological process, for example, proteolytic cleavage, or upon reaching an environment of a certain pH.

Methods of the invention can be used on any individual in need of such methods or individuals for whom practice of the methods is desired. These individuals may be any mammal including, but not limited to, humans, dogs, horses, cows, pigs, chickens and other commercially valuable or companion animals.

EXAMPLE 1

High fat-fed (58% kcal from fat, D12331, Research Diets), male SPRAGUE-DAWLEY® rats were implanted subcutaneously with 28-day osmotic pumps (Durect Corp.) delivering amylin (300 μg/kg/day), sibutramine (3 mg/kg/day), or vehicle (50% dimethyl sulfoxide (DMSO)). Low fat-fed rats (11% kcal from fat, D12329, Research Diets) were also implanted with pumps delivering vehicle. Food intake and body weight measurements were obtained weekly.

Rats were sacrificed by cardiac puncture under anesthesia. Triglyceride levels were measured on a COBAS Mira plasma analyzer (Roche), and leptin and insulin were assayed according to Linco Research rat RIA kits. Body composition was measured by chemical analysis (Covance Laboratories, Madison, WI).

Amylin was synthesized by Amylin Pharmaceuticals, Inc. by solid-phase chemistry, purified by HPLC (>98% purity, 84% peptide content), and characterized by amino acid analysis and LC/MS. Sibutramine was extracted from the drug product MERIDIA® using water as a solvent, purified by RP-HPLC (>98% purity), and characterized by NMR and LC/MS.

All data are represented as mean ± SEM. Analysis of variance was used to test for group differences.

The rats were fattened for 10 weeks prior to drug treatment. The high fat-fed rats were designated as obesity-prone (top 50% of weight gainers) or obesity-resistant (bottom 50%) based on the amount of weight gained through week 7. No difference between prone and resistant animals was observed for food consumption, body weight, or plasma metabolites in response to drug treatment; therefore, these groups were combined (Table 1, Figures 1A, 1B, 3A, 3B, and 3C).

Table 1

	Weekly Caloric Intake				Cummulative Body Weight			
Week	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Study 1								
Amylin 300 μg/kg/day	45*	14*	10*	10*	6*	7*	8*	8*
Sibutramine 3 mg/kg/day	45*	8*	-1	-3	6*	6*	6*	3
Study 2								
Amylin								
30 μg/kg/day	32*	17*	10	8	5*	7*	7*	7*
100 μg/kg/day	45*	19*	4	9	10*	10*	9*	8*
300 μg/kg/day	41*	15*	19*	19*	7*	8*	10*	12*

* Significantly different from high fat-fed controls.

In this study, an obesity-prone/obesity-resistant difference in drug interaction was found for protein weight in amylin-treated rats, and thus body composition parameters were measured separately in obesity-prone and obesity-resistant animals in each drug group (Figures 2A and 2B). In obesity-prone rats, there was an increase in protein in the amylin-treated group when compared to the control group (vehicle only).

EXAMPLE 2

This experiment was similar to that of Example 1, except that the study group consisted of high fat-fed rats implanted with pumps delivering three doses of amylin (30,

100, and 300 μg/kg/day) or vehicle. Figures 4A and 4B show that the effects of amylin on food intake and body weight are dose-dependent, with a reduction in body weight gain being observed at 30 μg/kg/day.

EXAMPLE 3

Lean, male Harlan SPRAGUE DAWLEY® (HSD) (Harlan 7012) rats were maintained on "standard chow" (~5% calories from fat). DIO (Levin; Charles River) male rats were maintained on Research Diets' 12266B chow (17% protein, 51% carbohydrate, 32% fat) for 6 weeks prior to the experiment, resulting in a weight gain of ~150 to 200 g/animal.

Rats were implanted subcutaneously with 28-day osmotic pumps containing either amylin (300 mg/kg/day; synthesized at Amylin Pharmaceuticals, Inc.) or vehicle (50% DMSO; control and pair-fed groups). Food intake and body weight were recorded daily (Figures 5, 6A, 6B, 7, 8A, and 8B). While amylin and vehicle-control rats always had *ad libitum* access to food, intake in the pair-fed control group was restricted to the amount consumed by the amylin-treated group.

On the final day of the experiment, rats were deeply anaesthetized and sacrificed by cardiac puncture. Plasma triglycerides, glucose, and cholesterol were measured on a COBAS Mira plasma Analyzer (Roche). Plasma leptin and insulin were measured using Linco Research kits. (See, Figures 9A-9F and 10A-10E.) Body composition was measured by chemical analysis (Covance Laboratories, Madison, WI). Fat pad weights of the epididymal, retroperitoneal, subcutaneous, and perirenal fat pads (all unilateral; analysis only done in DIO animals) were carefully dissected and weighed (Figures 12A-12D). In analyzing the tissue biochemistry, triglycerides were powdered under liquid N_2 and extracted in chloroform:methanol. 0.6% NaCl solution was then added and the tubes were vortexed, centrifuged, and the organic phase was transferred to glass scintillation vials and dried under a stream of N_2. Dried lipids were resuspended and triglycerides were quantified by enzymatic assay (Pointe Scientific, Inc.). Tissue glycogen was measured by the amyloglucosidase method. (See Figures 11A-11D.)

All data are represented as mean ± SEM. Analysis of variance (ANOVA) and Bonferroni post-hoc tests were used to test for group differences (SYSTAT® for Windows). A *P*-value <0.05 was considered significant. Graphs were generated using PRISM® 4 for Windows (Graphpad Software).

Results showed that amylin treatment and pair-feeding both induced a 12% reduction in body weight relative to vehicle controls in lean and DIO rats. Chronic infusion of amylin significantly changed body composition relative to pair-fed and/or vehicle animals.

Amylin-treated lean rats and pair-fed lean rats showed a significant reduction in weight gain compared to vehicle rats. Amylin-treated lean rats also had a lower percent body fat relative to pair-fed while the percent protein remained relatively constant, suggesting amylin may have a metabolic mechanism of action as well as the ability to reduce food intake.

Table 2

	Vehicle	Pair-fed	Amylin
Weight (g)	425.45	397.85*	392.25*
Fat (%)	8.3 ± 0.9	9.52 ± 1.2	7.2 ± 1.5†
Protein (%)	20.72 ± 0.69	20.62 ± 1.07	20.67 ± 0.74
Moisture (%)	66.68 ± 0.7	66.27 ± 0.7	67.57 ± 0.7†
Ash (%)	3.52 ± 0.15	3.1 ± 0.66	3.22 ± 0.32

* P <0.05, compared to vehicle.
† P <0.05, compared to pair-fed.

Amylin-treated DIO rats and pair-fed DIO rats showed a significant reduction in weight gain compared to vehicle rats. Amylin-treated DIO rats also showed a significant decrease in % body fat and a significant preservation or gain in percent protein. Again, this result suggests that amylin may have a metabolic as well as weight reducing effect.

Table 3

	Vehicle	Pair-fed	Amylin
Weight (g)	612.99	551.33*	548.94*
Fat (%)	33.4 ± 4.7	27.64 ± 5.7	24.3 ± 6.5*
Protein (%)	15.61 ± 1.37	16.85 ± 1.53	18.09 ± 1.68*
Moisture (%)	49.46 ± 2.6	53.93 ± 4.5	56.68 ± 4.4*
Ash (%)	1.34 ± 0.26	1.81 ± 0.59	1.65 ± 0.34

* P <0.05, compared to vehicle.

Also seen from this experiment is that reductions in body weight were not accompanied by alterations in liver or muscle triglycerides or in liver glycogen content. However, rats given amylin or pair-fed had significantly reduced muscle glycogen content. Further, reductions in body weight were generally accompanied by reductions in metabolites and plasma insulin and leptin.

AMYLIN and AMYLIN AGONISTS

Amylin is a 37 amino acid peptide hormone that is co-secreted with insulin from pancreatic ß-cells in response to nutrient stimuli. Human amylin has the following amino acid sequence:
Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr (SEQ ID NO:1), although the use of amylins from any species is contemplated.

Amylin agonists contemplated in the use of the invention include amylin agonist analogs. Amylin agonist analogs include those analogs and derivatives of amylin described in U.S. Patent Nos. 5,686,411, 6,114,304, and 6,410,511, which are herein incorporated by reference in their entirety. Amylin agonist analogs also include those compounds described in U.S. Provisional Application No. 60/543,275, filed on February 11, 2004, which is incorporated herein in its entirety. Amylin agonist analogs useful in

the invention may also include fragments of amylin such as those described in EP 289287, the contents of which are herein incorporated by reference.

Amylin agonist analogs may also be compounds having at least 60, 65, 70, 75, 80, 85, 90, 95, or 99% amino acid sequence identity to SEQ ID NO:1, as well as fragments thereof, having an amylin activity. "Amylin activity" as used herein includes the ability to bind to an amylin receptor. Amylin receptors and their use in methods for screening and assaying for amylin agonists are described in U.S. Patent No. 5,264,372, issued November 23, 1993, incorporated herein by reference. "Amylin activity" as used herein may also include any one or more of those amylin activities described in U.S. Patent Application No. 09/445,517, filed June 5, 1998, previously incorporated by reference.

Amylin agonist analogs include analogs and derivatives of amylin having insertions, extensions, deletions and/or substitutions in at least one or more amino acid positions of SEQ ID NO:1 and have amylin activity. The number of amino acid insertions, extensions, deletions, or substitutions may be at least 5, 10, 15, 20, 25, or 30. Insertions, extensions, or substitutions may be with other natural amino acids, synthetic amino acids, peptidomimetics, or other chemical compounds.

Also contemplated as amylin agonists are calcitonins, such as teleost calcitonins, and their analogs and derivatives, as well as calcitonin-gene-related peptides (CGRP) and their analogs and derivatives.

Methods of the invention contemplate the use of one or more of the compounds known as amylin, amylin agonist analog, or amylin agonist.

DOSAGE/FORMULATION

Amylin and amylin agonist (herein referred to as the "amylin compounds") may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. These pharmaceutical compounds may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington's Pharmaceutical Sciences by E. W. Martin. See also

Wang, Y. J. and Hanson, M. A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988), incorporated by reference.

Exemplary formulations for an amylin or amylin agonist can be found in U.S. Patent No. 6,410,511 and U.S. Patent Application No. 10/159,779, filed May 31, 2002, which are incorporated herein by reference.

In general, the amylin compounds may be formulated into a stable, safe pharmaceutical composition for administration to a patient. Pharmaceutical formulations contemplated for use in the methods of the invention may comprise approximately 0.01 to 1.0% (w/v), preferably 0.05 to 1.0%, of the amylin compound, approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer allowing a pH of the final composition of from about 3.0 to about 7.0; approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol tonicifier and, optionally, approximately 0.005 to 1.0% (w/v) of a preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol. Such a preservative is generally included if the formulated peptide is to be included in a multiple use product.

In a particular embodiment of the present invention, a pharmaceutical formulation of the present invention may contain a range of concentrations of amylin compounds, *e.g.*, between about 0.01% to about 98% w/w, or between about 1 to about 98% w/w, or preferably between 80% and 90% w/w, or preferably between about 0.01% to about 50% w/w, or more preferably between about 10% to about 25% w/w in this embodiment. A sufficient amount of water for injection may be used to obtain the desired concentration of solution.

Additional tonicifying agents such as sodium chloride, as well as other known excipients, may also be present, if desired. It is preferred, however, if such excipients maintain the overall tonicity of the amylin compounds. An excipient may be included in the presently described formulations at various concentrations. For example, an excipient may be included in the concentration range from about 0.02% to about 20% w/w, preferably between about 0.02% and 0.5% w/w, about 0.02% to about 10% w/w, or about

1 % to about 20% w/w. In addition, similar to the present formulations themselves, an excipient may be included in solid (including powdered), liquid, semi-solid or gel form.

The pharmaceutical formulations may be composed in various forms, *e.g.*, solid, liquid, semisolid or liquid. The term "solid", as used herein, is meant to encompass all normal uses of this term including, for example, powders and lyophilized formulations. The presently described formulations may be lyophilized.

The terms buffer, buffer solution and buffered solution, when used with reference to hydrogen-ion concentration or pH, refer to the ability of a system, particularly an aqueous solution, to resist a change of pH on adding acid or alkali, or on dilution with a solvent. Characteristic of buffered solutions, which undergo small changes of pH on addition of acid or base, is the presence either of a weak acid and a salt of the weak acid, or a weak base and a salt of the weak base. An example of the former system is acetic acid and sodium acetate. The change of pH is slight as long as the amount of hydronium or hydroxyl ion added does not exceed the capacity of the buffer system to neutralize it.

As described herein, a variety of liquid vehicles are suitable for use in the present peptide formulations, for example, water or an aqueous/organic solvent mixture or suspension.

The stability of a peptide formulation of the present invention is enhanced by maintaining the pH of the formulation in the range of about 3.0 to about 7.0 when in liquid form. Preferably, the pH of the formulation is maintained in the range of about 3.5 to 5.0, or about 3.5 to 6.5, most preferably from about 3.7 to 4.3, or about 3.8 to 4.2. A frequently preferred pH may be about 4.0. While not seeking to be bound by this theory, it is presently understood that where the pH of the pharmaceutical formulation exceeds 5.5, chemical degradation of the peptide may be accelerated such that the shelf life is less than about two years.

The buffer used in the practice of the present invention is an acetate buffer (preferably at a final formulation concentration of from about 1-5 to about 60 mM), phosphate buffer (preferably at a final formulation concentration of from about 1-5 to

about to about 30 mM) or glutamate buffer (preferably at a final formulation concentration of from about 1-5 to about to about 60 mM). The most preferred buffer is acetate (preferably at a final formulation concentration of from about 5 to about 30 mM).

A stabilizer may be included in the present formulation but, and importantly, is not necessarily needed. If included, however, a stabilizer useful in the practice of the present invention is a carbohydrate or a polyhydric alcohol. A suitable stabilizer useful in the practice of the present invention is approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol. The polyhydric alcohols and carbohydrates share the same feature in their backbones, i.e., -CHOH-CHOH-, which is responsible for stabilizing the proteins. The polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols (PEGs). These compounds are straight-chain molecules. The carbohydrates, such as mannose, ribose, sucrose, fructose, trehalose, maltose, inositol, and lactose, on the other hand, are cyclic molecules that may contain a keto or aldehyde group. These two classes of compounds have been demonstrated to be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include: galactose, arabinose, lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient (if this is a desirable property), *i.e.*, the carbohydrate is not metabolized to form unacceptably large concentrations of glucose in the blood.

Preferably, if a stabilizer is included, the amylin compound is stabilized with a polyhydric alcohol such as sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the preferred polyhydric alcohol. Another useful feature of the lyophilized formulations of the present invention is the maintenance of the tonicity of the lyophilized formulations described herein with the same formulation component that serves to maintain their stability. Mannitol is the preferred polyhydric alcohol used for this purpose.

The United States Pharmacopeia (USP) states that anti-microbial agents in bacteriostatic or fungistatic concentrations must be added to preparations contained in multiple dose containers. They must be present in adequate concentration at the time of use to prevent the multiplication of microorganisms inadvertently introduced into the preparation while withdrawing a portion of the contents with a hypodermic needle and syringe, or using other invasive means for delivery, such as pen injectors. Antimicrobial agents should be evaluated to ensure compatibility with all other components of the formula, and their activity should be evaluated in the total formula to ensure that a particular agent that is effective in one formulation is not ineffective in another. It is not uncommon to find that a particular antimicrobial agent will be effective in one formulation but not effective in another formulation.

A preservative is, in the common pharmaceutical sense, a substance that prevents or inhibits microbial growth and may be added to pharmaceutical formulations for this purpose to avoid consequent spoilage of the formulation by microorganisms. While the amount of the preservative is not great, it may nevertheless affect the overall stability of the peptide.

While the preservative for use in the pharmaceutical compositions can range from 0.005 to 1.0% (w/v), the preferred range for each preservative, alone or in combination with others, is: benzyl alcohol (0.1-1.0%), or m-cresol (0.1-0.6%), or phenol (0.1-0.8%) or combination of methyl (0.05-0.25%) and ethyl or propyl or butyl (0.005%-0.03%) parabens. The parabens are lower alkyl esters of para-hydroxybenzoic acid.

An exemplary amylin agonist analog pramlintide, human ^{25,28,29}Pro-amylin, does not have a tendency to adsorb onto the glass in a glass container when in a liquid form, therefore, a surfactant is not required to further stabilize the pharmaceutical formulation. However, with regard to amylin compounds that do have such a tendency when in liquid form, a surfactant may be used in their formulation. These formulations may then be lyophilized. Surfactants can cause denaturation of protein, both of hydrophobic disruption and by salt bridge separation. Relatively low concentrations of surfactant may exert a potent denaturing activity, because of the strong interactions between surfactant

moieties and the reactive sites on proteins. However, judicious use of this interaction can stabilize proteins against interfacial or surface denaturation. Surfactants which could further stabilize the peptide may optionally be present in the range of about 0.001 to 0.3% (w/v) of the total formulation and include polysorbate 80 (i.e., polyoxyethylene(20) sorbitan monooleate), CHAPS® (i.e., 3-[(3-cholamidopropyl) dimethylammonio] 1-propanesulfonate), Brij® (e.g., Brij 35, which is (polyoxyethylene (23) lauryl ether), poloxamer, or another non-ionic surfactant.

It may also be desirable to add sodium chloride or other salt to adjust the tonicity of the pharmaceutical formulation, depending on the tonicifier selected. However, this is optional and depends on the particular formulation selected. Parenteral formulations are preferably isotonic or substantially isotonic.

A preferred vehicle for parenteral products is water. Water of suitable quality for parenteral administration can be prepared either by distillation or by reverse osmosis. Water for injection is the preferred aqueous vehicle for use in the pharmaceutical formulations.

It is possible that other ingredients may be present in the pharmaceutical formulations. Such additional ingredients may include, e.g., wetting agents, emulsifiers, oils, antioxidants, bulking agents, tonicity modifiers, chelating agents, metal ions, oleaginous vehicles, proteins (e.g., human serum albumin, gelatin or proteins) and a zwitterion (e.g., an amino acid such as betaine, taurine, arginine, glycine, lysine and histidine). Additionally, polymer solutions, or mixtures with polymers provide the opportunity for controlled release of the peptide. Such additional ingredients, of course, should not adversely affect the overall stability of the pharmaceutical formulation of the present invention.

Containers are also an integral part of the formulation of an injection and may be considered a component, for there is no container that is totally inert, or does not in some way affect the liquid it contains, particularly if the liquid is aqueous. Therefore, the selection of a container for a particular injection must be based on a consideration of the composition of the container, as well as of the solution, and the treatment to which it will

be subjected. Adsorption of the peptide to the glass surface of the vial can also be minimized, if necessary, by use of borosilicate glass, for example, Wheaton Type I borosilicate glass #33 (Wheaton Type I-33) or its equivalent (Wheaton Glass Co.). Other vendors of similar borosilicate glass vials and cartridges acceptable for manufacture include Kimbel Glass Co., West Co., Bünder Glas GMBH and Forma Vitrum. The biological and chemical properties of amylin may be stabilized by formulation and lyophilization in a Wheaton Type I-33 borosilicate serum vial to a final concentration of 0.1 mg/ml and 10 mg/ml of amylin in the presence of 5% mannitol, and 0.02% Tween 80.

In order to permit introduction of a needle from a hypodermic syringe into a multiple-dose vial and provide for resealing as soon as the needle is withdrawn, the open end of each vial is preferably sealed with a rubber stopper closure held in place by an aluminum band.

Stoppers for glass vials, such as, West 4416/50, 4416/50 (Teflon faced) and 4406/40, Abbott 5139 or any equivalent stopper can be used as the closure for pharmaceutical for injection. These stoppers are compatible with the peptide as well as the other components of the formulation. The inventors have also discovered that these stoppers pass the stopper integrity test when tested using patient use patterns, e.g., the stopper can withstand at least about 100 injections. Alternatively, the peptide can be lyophilized in to vials, syringes or cartridges for subsequent reconstitution. Liquid formulations of the present invention can be filled into one or two chambered cartridges, or one or two chamber syringes.

The manufacturing process for the above liquid formulations generally involves compounding, sterile filtration and filling steps. The compounding procedure involves dissolution of ingredients in a specific order (preservative followed by stabilizer/tonicity agents, buffers and peptide) or dissolving at the same time.

Alternative formulations, *e.g.*, non-parenteral, may not require sterilization. However, if sterilization is desired or necessary, any suitable sterilization process can be used in developing the peptide pharmaceutical formulation of the present invention. Typical sterilization processes include filtration, steam (moist heat), dry heat, gases (e.g.,

ethylene oxide, formaldehyde, chlorine dioxide, propylene oxide, beta-propiolacctone, ozone, chloropicrin, peracetic acid methyl bromide and the like), exposure to a radiation source, and aseptic handling. Filtration is the preferred method of sterilization for liquid formulations of the present invention. The sterile filtration involves filtration through 0.45 μm and 0.22 μm (1 or 2) which may be connected in series. After filtration, the solution is filled into appropriate vials or containers.

The liquid pharmaceutical formulations of the present invention are intended for parenteral administration. Suitable routes of administration include intramuscular, intravenous, subcutaneous, intradermal, mucosal, intraarticular, intrathecal and the like. These routes include, but are not limited to, oral, nasal, sublingual, pulmonary and buccal routes that may include administration of the amylin compound in liquid, semi-solid or solid form. Administration via some routes require substantially more amylin compound to obtain the desired biological effects due to decreased bioavailability compared to parenteral delivery. In addition, parenteral controlled release delivery can be achieved by forming polymeric microcapsules, matrices, solutions, implants and devices and administering them parenterally or by surgical means. Examples of controlled release formulations are described in U.S. Patent Nos. 6,368,630, 6,379,704, and 5,766,627, which are incorporated herein by reference. These dosage forms may have a lower bioavailability due to entrapment of some of the peptide in the polymer matrix or device. See e.g., U.S. Pat. Nos. 6,379,704, 6,379,703, and 6,296,842.

The amylin compounds may be provided in dosage unit form. Therapeutically effective amounts of the amylin compound for affecting body composition will vary with many factors including the age and weight of the patient, the patient's physical condition, their use in combination with other treatments, the ultimate goal that is to be achieved, such as overall weight loss and/or maintaining or increasing lean body mass, as well as other factors.

However, typical doses may contain from a lower limit of about 1 μg, 5 μg, 10 μg, 50 μg to 100 μg to an upper limit of about 100 μg, 500 μg, 1 mg, 5 mg, 10 mg, 50 mg, or 100 mg of the pharmaceutical compound per day. Also contemplated are other

dose ranges such as 0.1 μg to 1 mg of the compound per dose. The doses per day may be delivered in discrete unit doses, provided continuously in a 24 hour period or any portion of that the 24 hours. The number of doses per day may be from 1 to about 4 per day, although it could be more. Continuous delivery can be in the form of a continuous infusion. Exemplary doses and infusion rates include from 0.005 nmol/kg to about 20 nmol/kg per discrete dose or from about 0.01/pmol/kg/min to about 10 pmol/kg/min in a continuous infusion. These doses and infusions can be delivered by intravenous administration (i.v.) or subcutaneous administration (s.c.). Exemplary total dose/delivery of the pharmaceutical composition given i.v. may be about 2 μg to about 8 mg per day, whereas total dose/delivery of the pharmaceutical composition given s.c may be about 6 μg to about 16 mg per day.

While the foregoing description discloses the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the present invention encompasses all of the usual variations, adaptations, or modifications as being within the scope of the claimed invention.

Claims:

1. A method for reducing body fat or preventing body fat gain in an overweight or obese individual, while maintaining body protein comprising:

administering to the individual an amylin or amylin agonist; thereby, reducing body fat or preventing body fat gain while maintaining body protein.

ABSTRACT

Methods for affecting body composition include the use of amylin or amylin agonist(s). Total body weight may be reduced, maintained or even increased; however, the body fat is reduced or body fat gain is prevented, while lean body mass is maintained or increased.

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Figure 1. Amylin, but not Sibutramine, Produces Sustained Decreases in Food Consumption and Body Weight Gain During Chronic Infusion

Fig. 1A

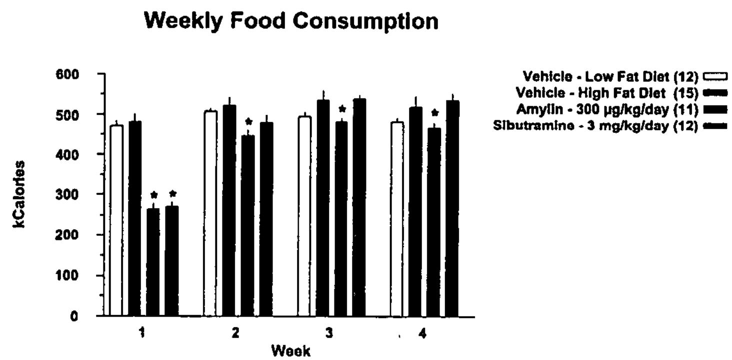
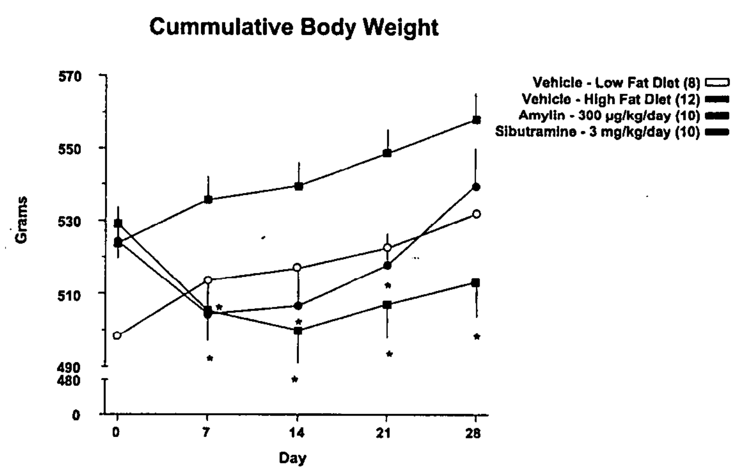


Fig. 1B



* P< 0.05 compared to Vehicle - High Fat Diet group.

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Figure 2. Both Amylin and Sibutramine Prevent the Increase in Body Fat Induced by a High Fat Diet

Fig. 2A

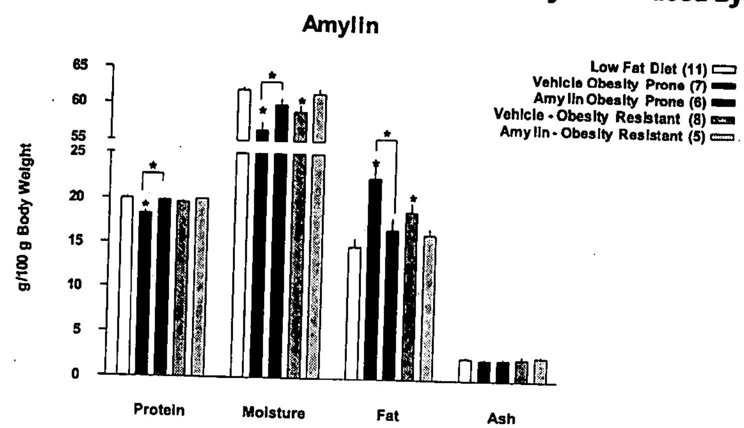
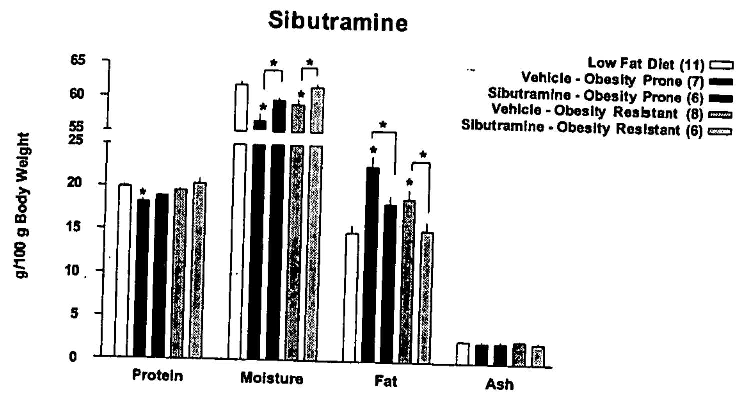


Fig. 2B



* $P<0.05$ compared to the Low Fat Diet group unless otherwise noted.

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Figure 3. Amylin Reduces Leptin and Insulin Levels; Sibutramine Reduces Insulin Levels

Fig. 3A

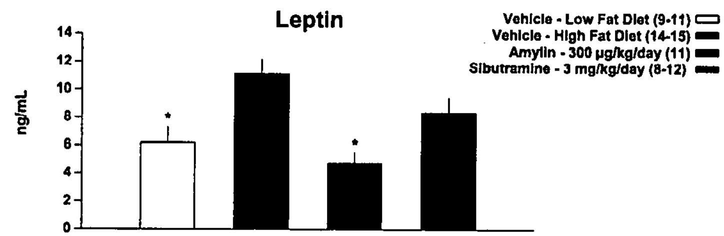


Fig. 3B

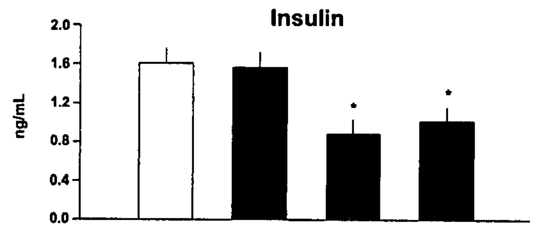
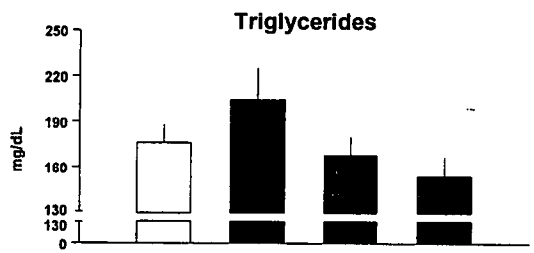


Fig. 3C



* P<0.05 compared to Vehicle - High Fat Diet group.

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Figure 4. Amylin dose-dependently decreases food consumption and body weight gain, with a significant reduction in body weight gain observed at 30 μg/kg/day

Fig. 4A

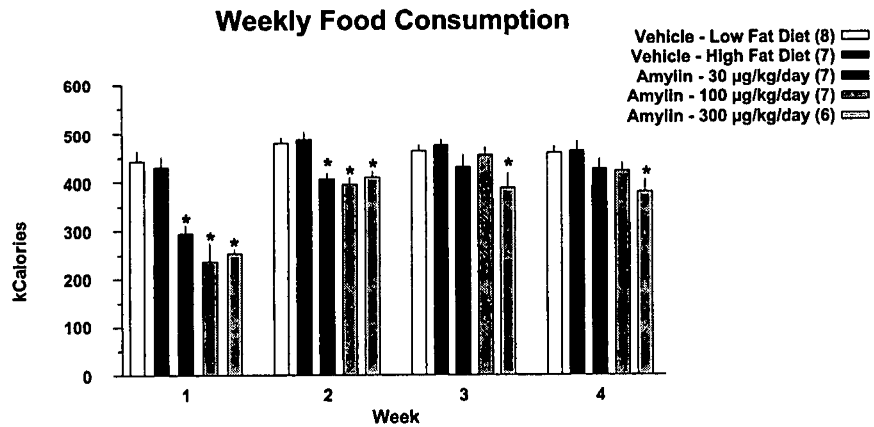
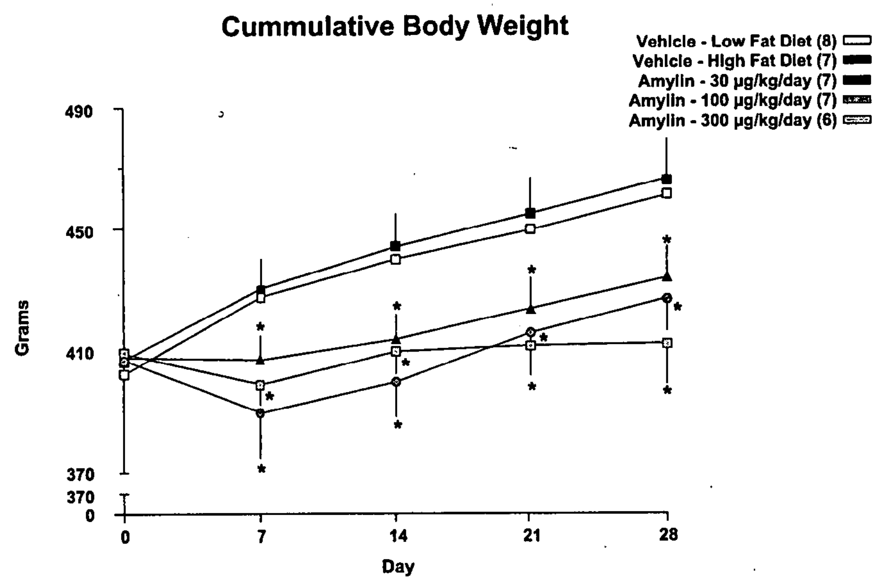


Fig. 4B



* $P<0.05$ compared to Vehicle - High Fat Diet group.

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Amylin Decreased Food Intake in Lean, Male HSD Rats

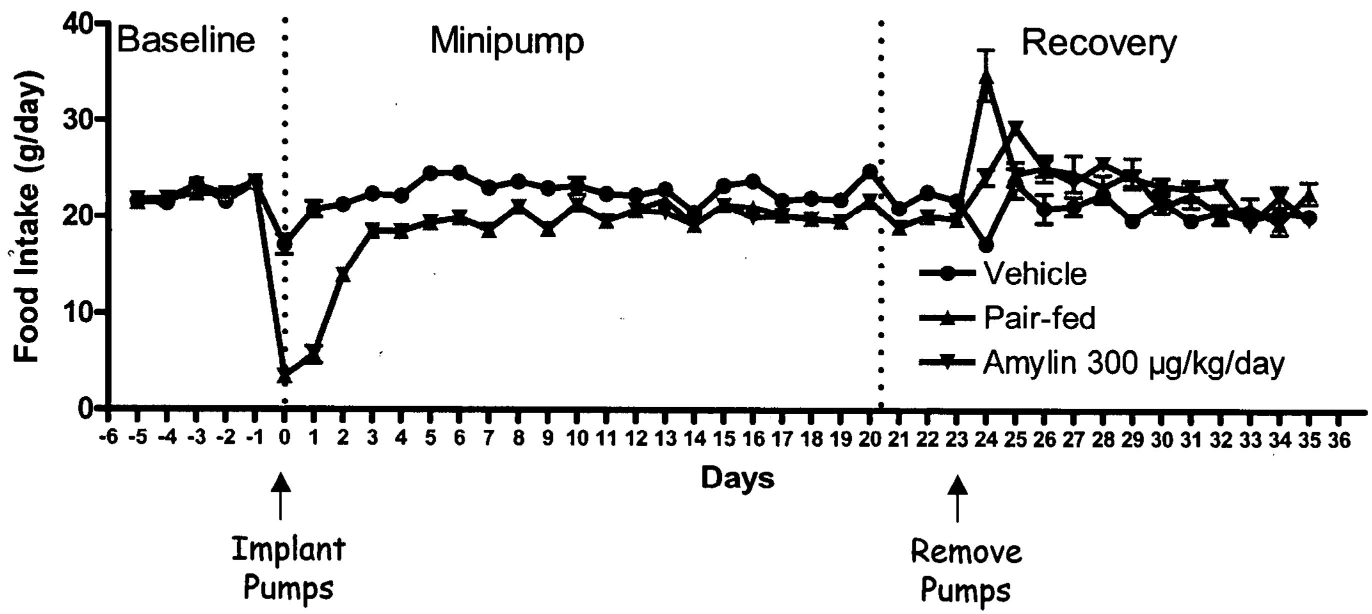

Fig. 5

Amylin Slowed Body Weight Gain in Lean, Male HSD Rats

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Fig. 6A

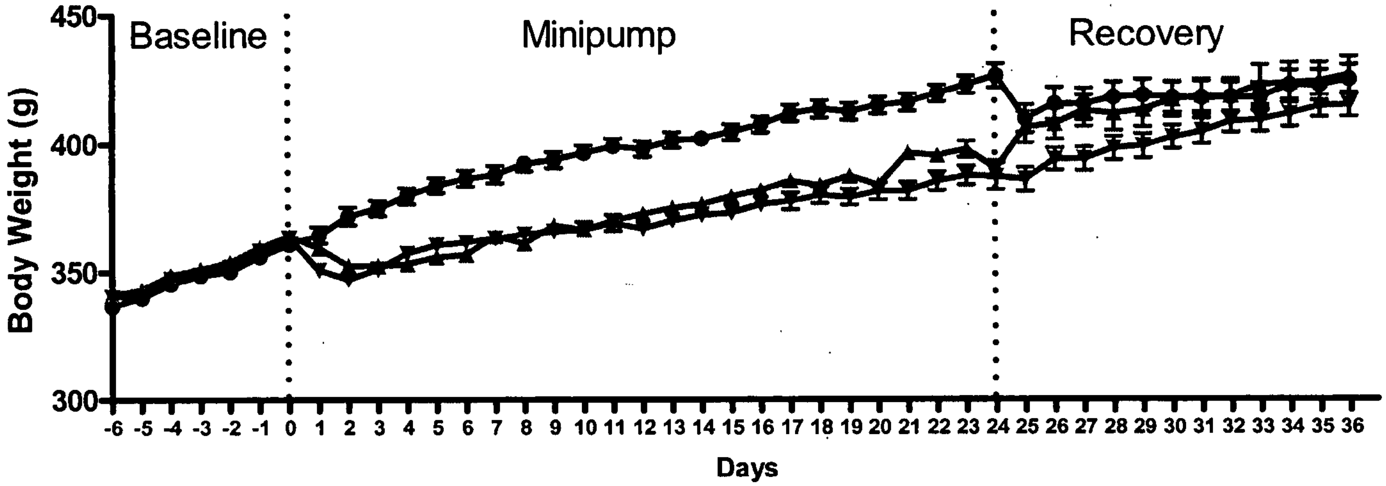
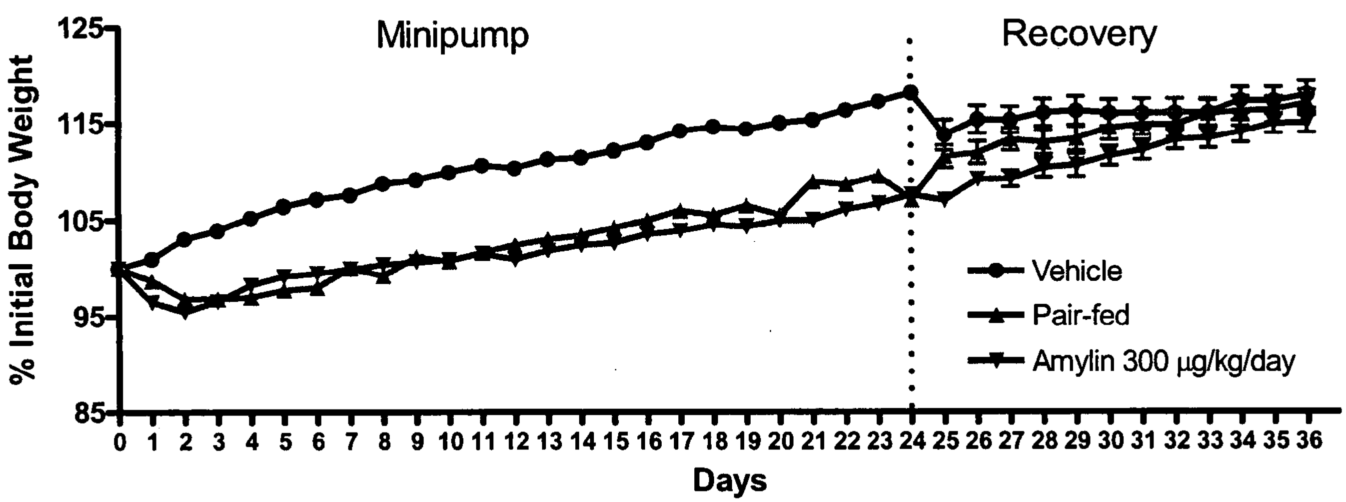


Fig. 6B



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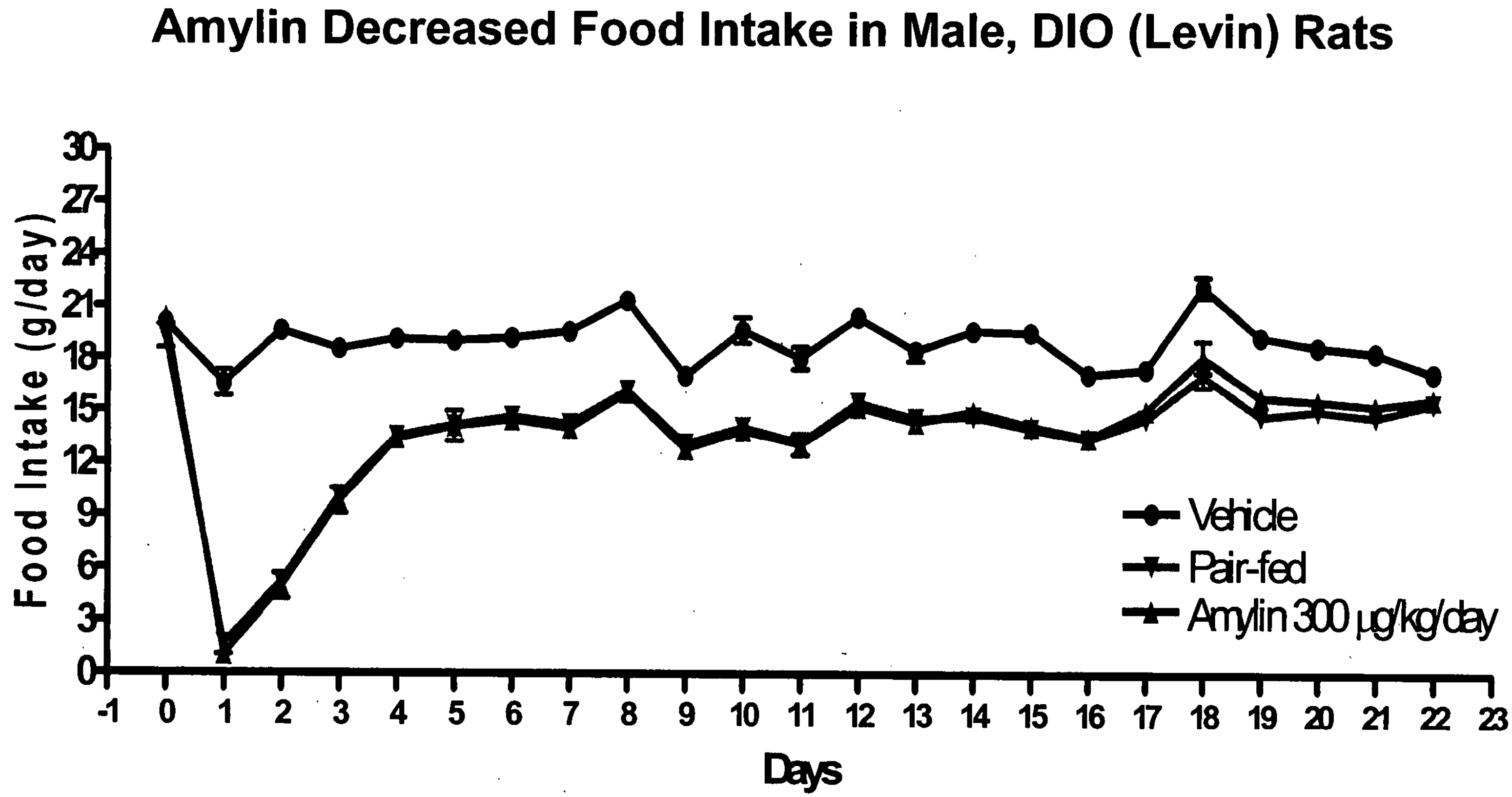

Fig. 7

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Amylin Slowed Body Weight Gain in Male, DIO (Levin) Rats

Fig. 8A

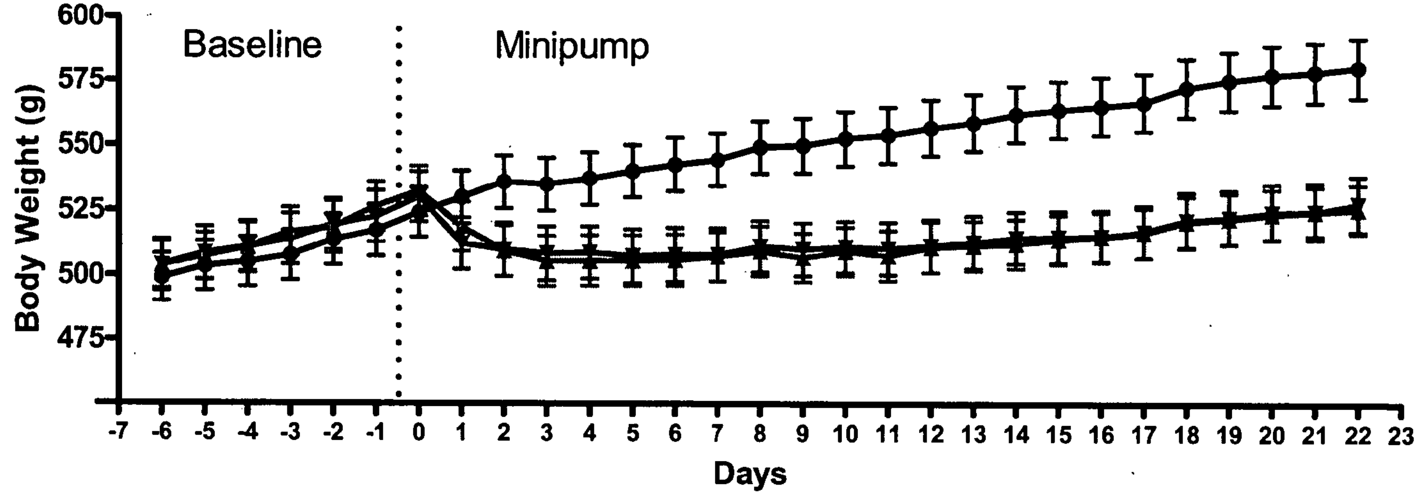
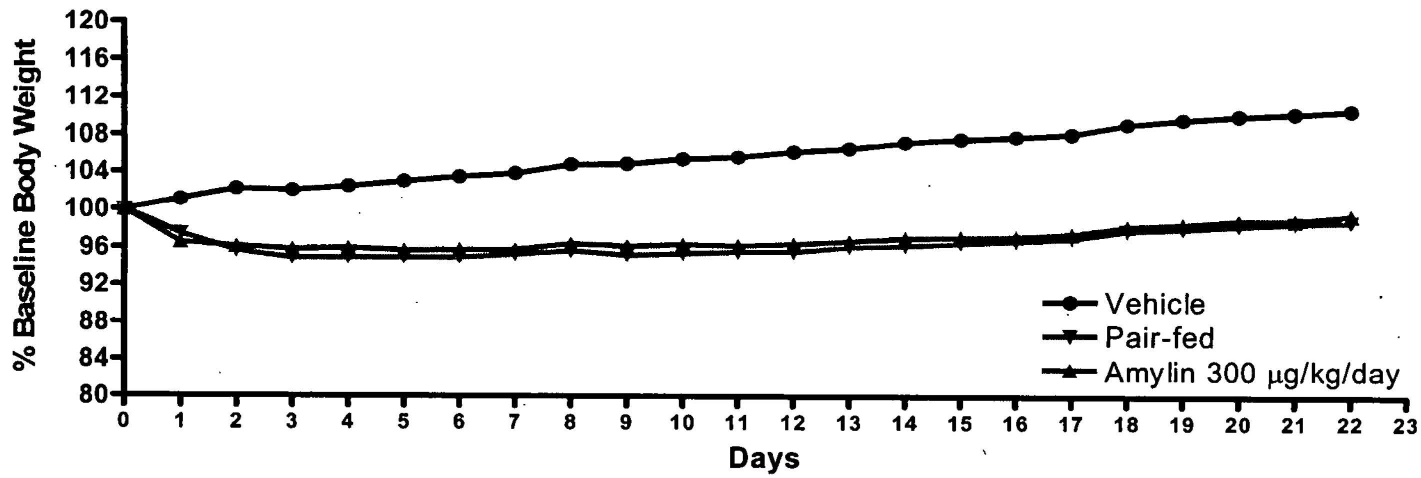


Fig. 8B



Plasma Parameters and Liver Triglycerides in Lean HSD, Male Rats

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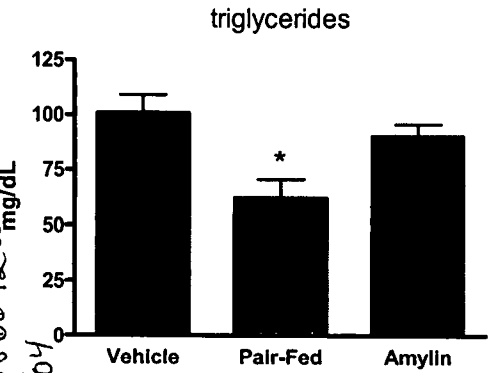

Fig. 9A

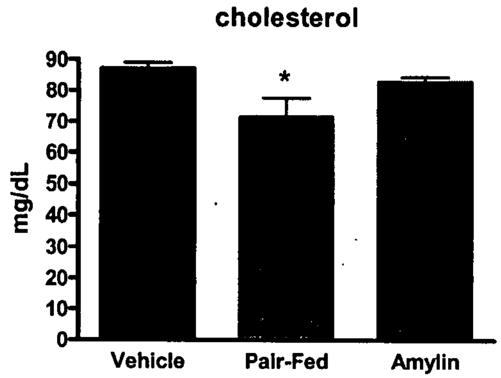

Fig. 9B

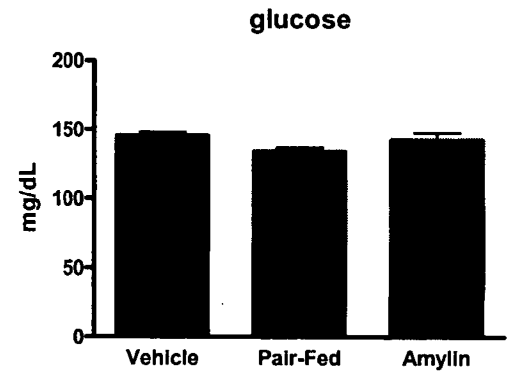

Fig. 9C

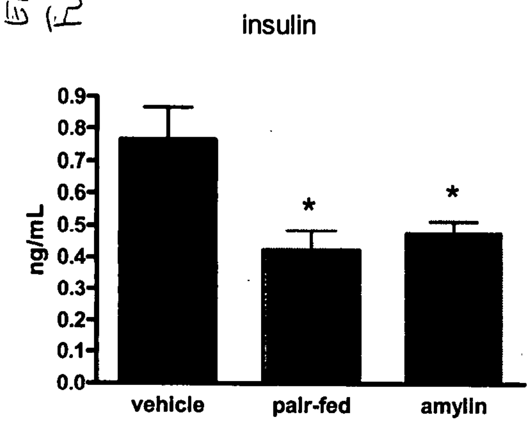

Fig. 9D

* P <0.05, compared to vehicle.

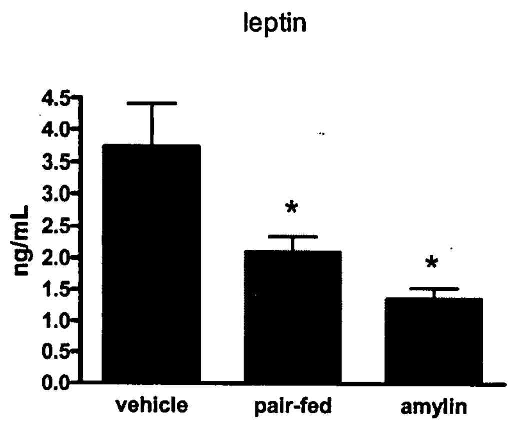

Fig. 9E

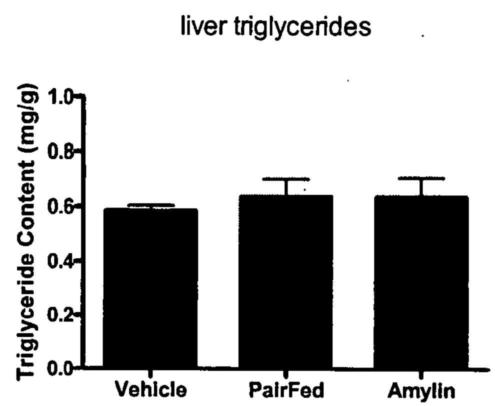

Fig. 9F

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Amylin and Pair-feeding Induced Changes in Plasma Parameters in DIO (Levin) rats

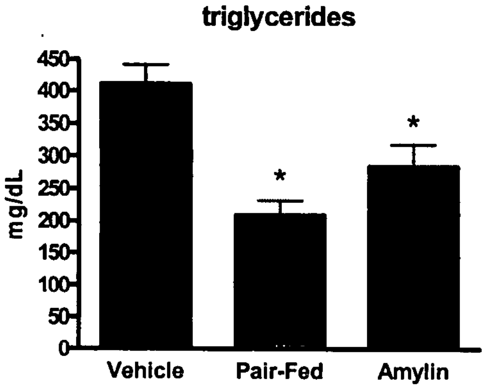

Fig. 10A

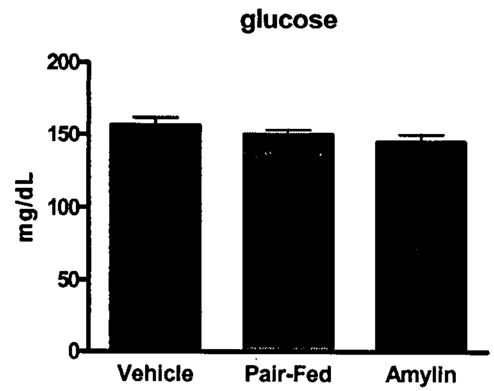

Fig. 10B

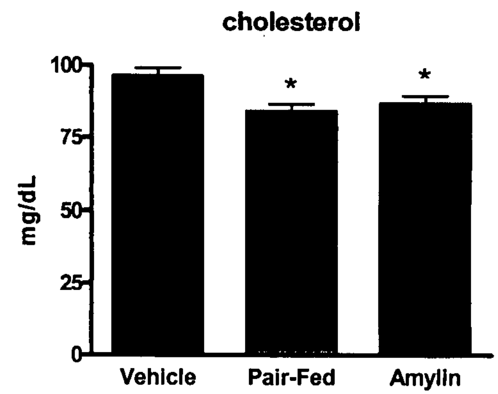

Fig. 10C

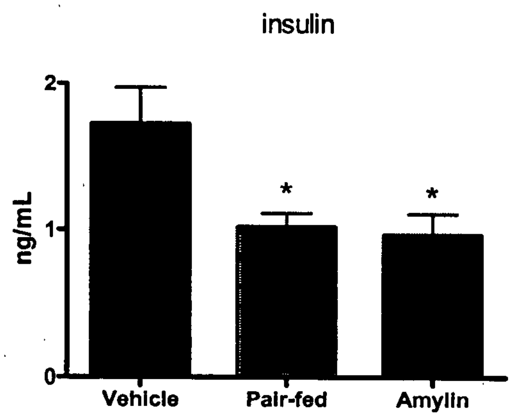

Fig. 10D

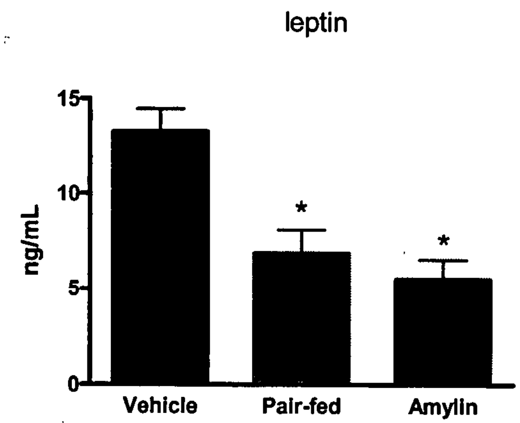

Fig. 10E

* $P < 0.05$, compared to vehicle.

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Tissue Biochemistry in Male, DIO (Levin) Rats

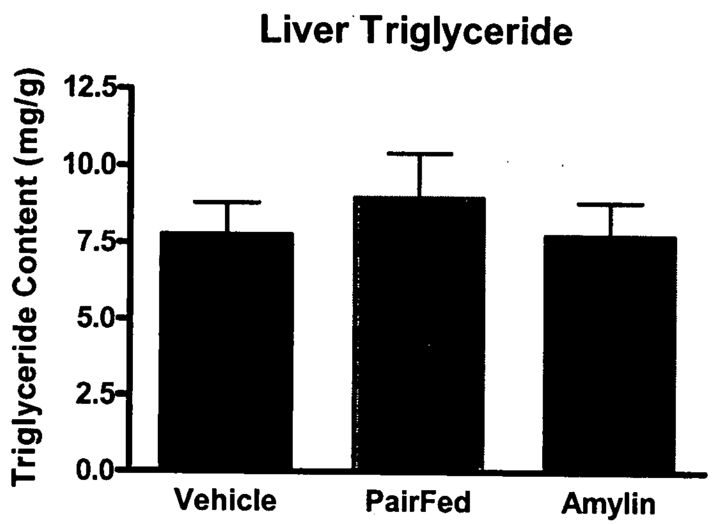

Fig. 11A

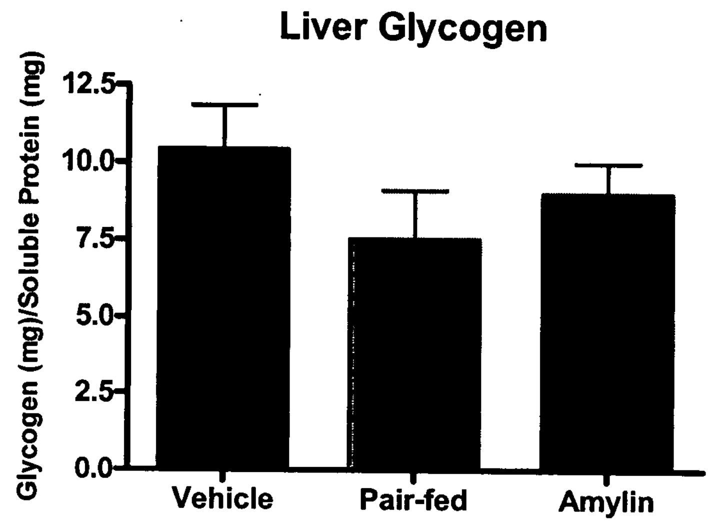

Fig. 11B

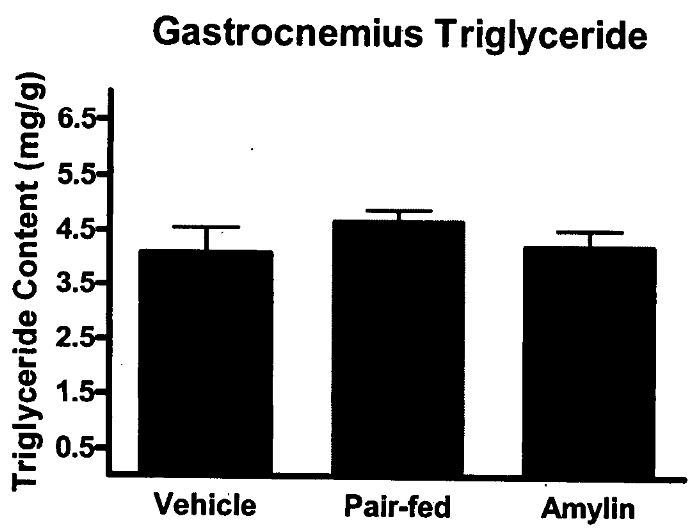

Fig. 11C

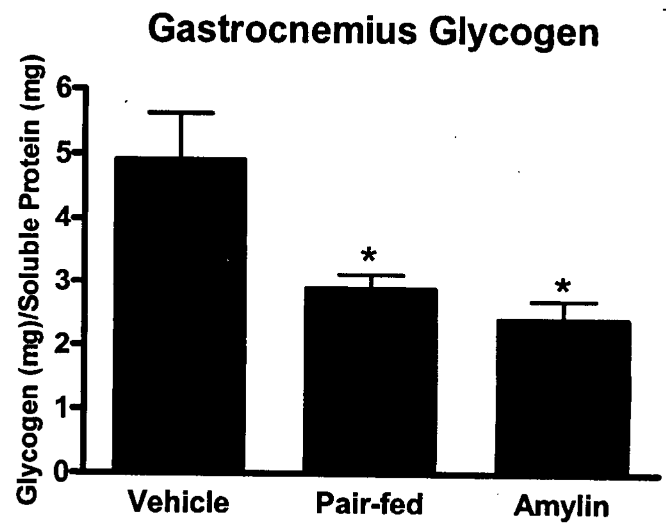

Fig. 11D

* P <0.05, compared to vehicle.

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Weights of Selected Fat Pads (as % of total body weight)

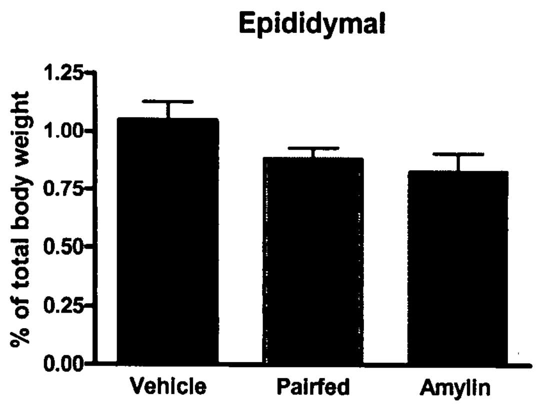

Fig. 12A

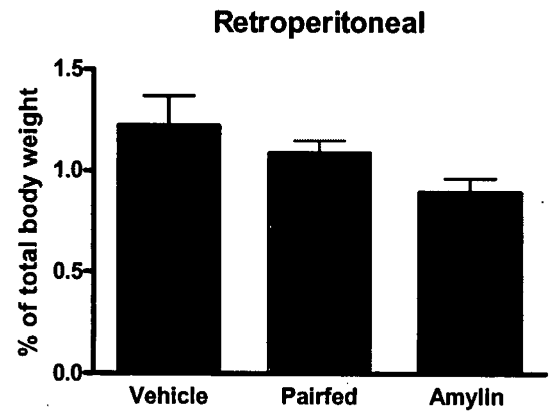

Fig. 12B

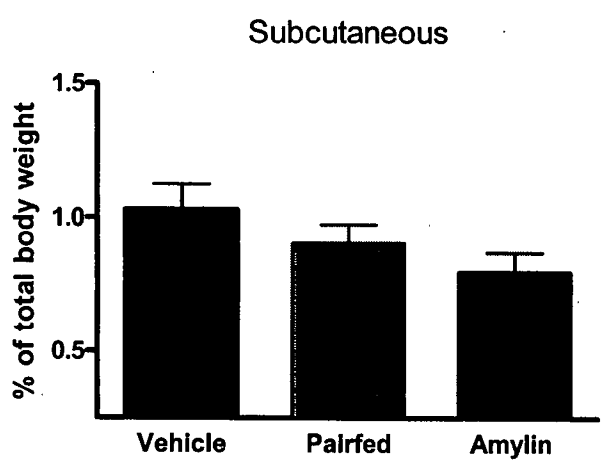

Fig. 12C

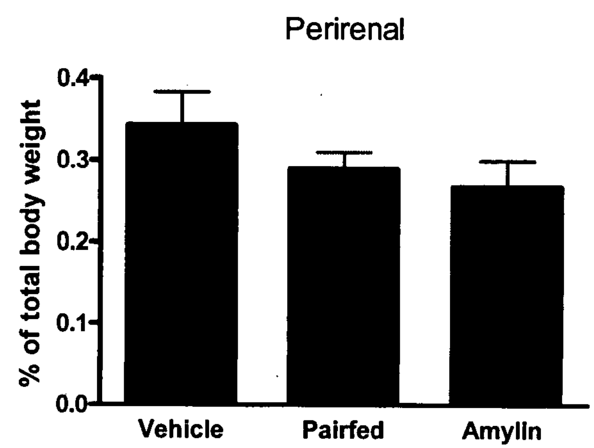

Fig. 12D